Antibody Characterization Report for Peroxiredoxin-1

YCharOS Antibody Characterization Report

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Target:

Protein name: Peroxiredoxin-1

Alternative protein names: PAG, NKEF-A

Uniprot: Q06830

Gene name: *PRDX1*

Alternative gene names: *PAGA, PAGB, TDPX2*

This report guides researchers to select the most appropriate antibodies for Peroxiredoxin-1. We used an antibody characterization pipeline[1] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Peroxiredoxin-1 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. U2OS was selected based on evidence of appropriate Peroxiredoxin-1 protein expression determined through public proteomics databases, namely PaxDB[2] and DepMap[3]. U2OS was modified with CRISPR/Cas9 to knockout[4] the corresponding *PRDX1*.

The authors do not provide an assessment of the quality of the tested antibodies as their respective performances are limited to our finite experimental conditions. The readers should interpret the present findings based on their own scientific expertise. The authors acknowledge that an antibody that demonstrates specificity in the stated test conditions can be suboptimal in a different experimental format or in cell lines that differ from those directly tested here.

1 Laflamme, C. *et al.* Implementation of an antibody characterization procedure and application to the major ALS/FTD disease gene C9ORF72. *Elife* **8**, doi:10.7554/eLife.48363 (2019).

2 Wang, M., Herrmann, C. J., Simonovic, M., Szklarczyk, D. & von Mering, C. Version 4.0 of PaxDb: Protein abundance data, integrated across model organisms, tissues, and cell-lines. *Proteomics* **15**, 3163-3168, doi:10.1002/pmic.201400441 (2015).

3 Nusinow, D. P. *et al.* Quantitative Proteomics of the Cancer Cell Line Encyclopedia. *Cell* **180**, 387-402 e316, doi:10.1016/j.cell.2019.12.023 (2020).

4 Nicouleau, Michael, Pimentel, Luisa, Shlaifer, Irlna & Durcan, Thomas M. Generation of Knockout Cell Lines Using CRISPR-Cas9 and ddPCR Technology. (2020). doi:10.5281/zenodo.3875777

Table 1: Summary of the Peroxiredoxin-1 antibodies tested

Company	Catalog number	Lot number	RRID	Clonality	Clone ID	Host	Concentration (µg/µl)
Proteintech	15816-1-AP	not provided	AB_2170318	polyclonal	-	rabbit	0.14
Proteintech	66820-1-Ig	not provided	AB_2882163	monoclonal	2B2A2	mouse	1.0
Thermo	LF-MA0073	VJ3101923	AB_1069882	monoclonal	6E5	mouse	not provided
Thermo	PA3-750	VA299223	AB_2168166	polyclonal	-	rabbit	not provided
Bio-Techne	AF3488	XLO0318011	AB_2170320	polyclonal	-	goat	0.2
Bio-Techne	MAB3488	CDBP0316021	AB_2170319	monoclonal	477609	mouse	0.5
GeneTex	GTX101705	39577	AB_1241175	polyclonal	-	rabbit	10.0
GeneTex	GTX113793	40135	AB_2037651	polyclonal	-	rabbit	0.57
Abcam	ab41906	GR3226661-2	AB_2284360	polyclonal	-	rabbit	1.0
Abcam	ab109498	GR48348-14	AB_10865914	recombinant-mono	EPR5433	rabbit	0.18
Abcam	ab109506	GR-43632-12	AB_10864539	recombinant-mono	EPR5434	rabbit	0.05
Santa-Cruz	sc-59656	H1720	AB_2168191	monoclonal	2A4	mouse	not provided

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
Montreal Neurological Institute	-	CVCL_0042	U2OS	WT
Montreal Neurological Institute	-	CVCL_A4GN	U2OS	*PRDX1* KO

Figure 1: Analysis of Peroxiredoxin-1 antibodies by immunoblot.

Lysates of U2OS (WT and *PRDX1* KO) were prepared and 10 µg of lysates were processed for immunoblot with the indicated Peroxiredoxin-1 antibodies. The Ponceau stained transfers associated with each blot are shown. Antibody dilution used: 15816-1-AP at 1/5000; 66820-1-Ig at 1/5000; LF-MA0073 at 1/5000; PA3-750 at 1/1000; AF3488 at 1/1000; MAB3488 at 1/1000; GTX101705 at 1/1000; GTX113793 at 1/1000; ab41906 at 1/1000; ab109498 at 1/5000; ab109506 at 1/5000; sc-59656 at 1/1000. Predicted band size: 22 kDa.

Figure 2: Analysis of Peroxiredoxin-1 antibodies by immunoprecipitation.

Lysates were prepared and IP was performed using 1.0 µg of the indicated Peroxiredoxin-1 antibodies pre-coupled to either protein G or protein A Sepharose beads. The antibody concentration for both LF-MA0073 and PA3-750 is not provided and 1.0 µl was used for the IP. Samples were washed and processed for immunoblot with the indicated Peroxiredoxin-1 antibody. For immunoblot, AF3488 and MAB3488 were used at 1/10000 and 1/5000, respectively. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate; HC=antibody heavy chain; LC= antibody light chain.

Figure 3: Analysis of Peroxiredoxin-1 antibodies by immunofluorescence.

Parental and *PRDX1* KO cells were transfected with a GFP or mCherry plasmid, respectively. Parental and KO cells were mixed and plated to a 1:1 ratio on coverslips. Cells were stained with the indicated Peroxiredoxin-1 antibodies and with the corresponding Alexa-fluor 647 coupled secondary antibody. Acquisition of the green (parental), red (KO) and far-red (antibody staining) channels was performed. Parental and KO cells are outlined with green and red dashed line, respectively. Grayscale images of the far-red channel are shown. Representative images are shown. Bars = 40 µm. Antibody dilution used:15816-1-AP at 1/500; 66820-1-Ig at 1/1000; LF-MA0073 at 1/1000; PA3-750 at 1/1000; AF3488 at 1/500; MAB3488 at 1/50000; sc-59656 at 1/10000; GTX101705 at 1/1000; GTX113793 at 1/1000; ab41906 at 1/1000; ab109498 at 1/500; ab109506 at 1/250.

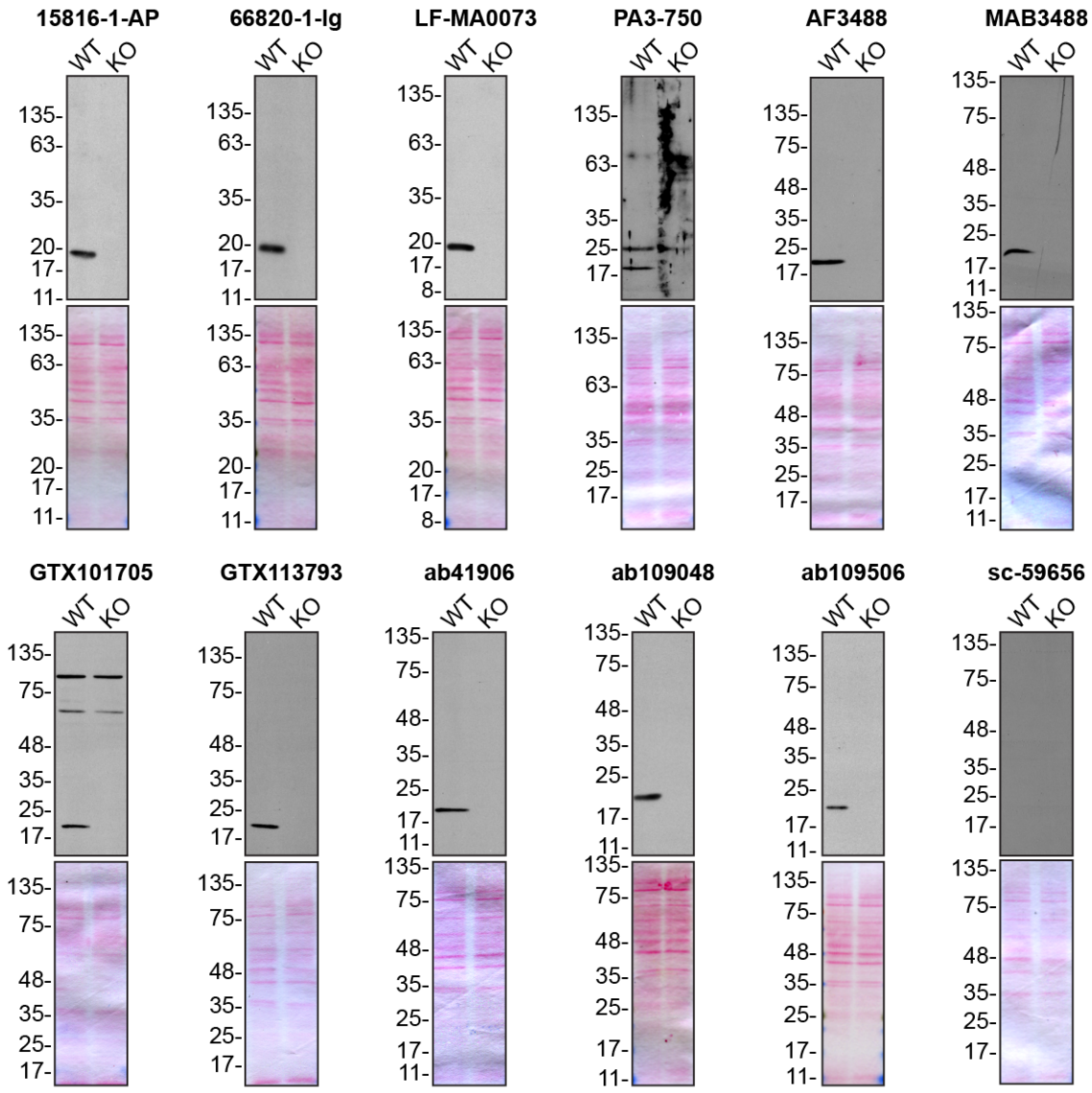

Figure 1: Peroxiredoxin-1 antibody screening by immunoblot

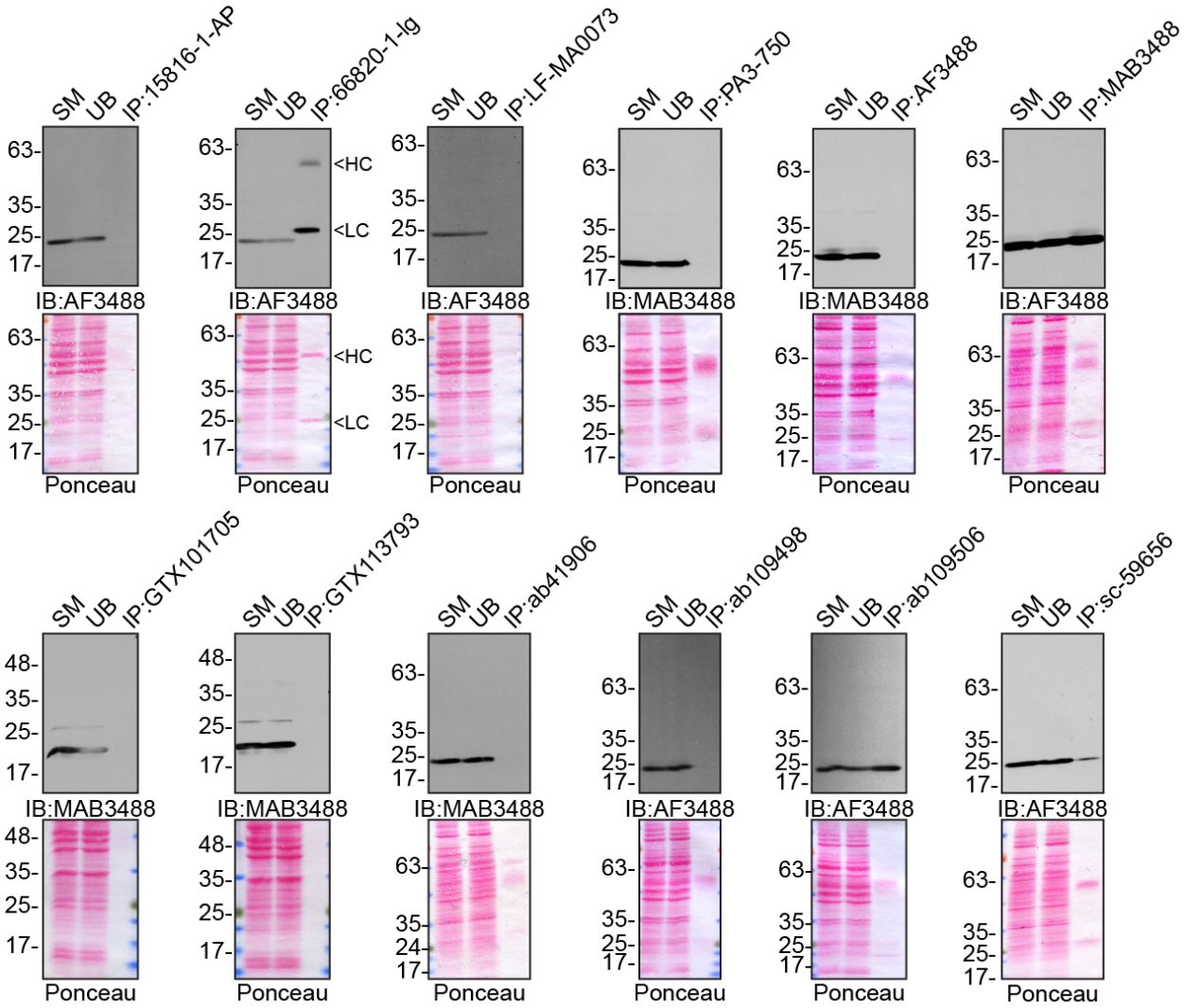

Figure 2: Peroxiredoxin-1 antibody screening by immunoprecipitation

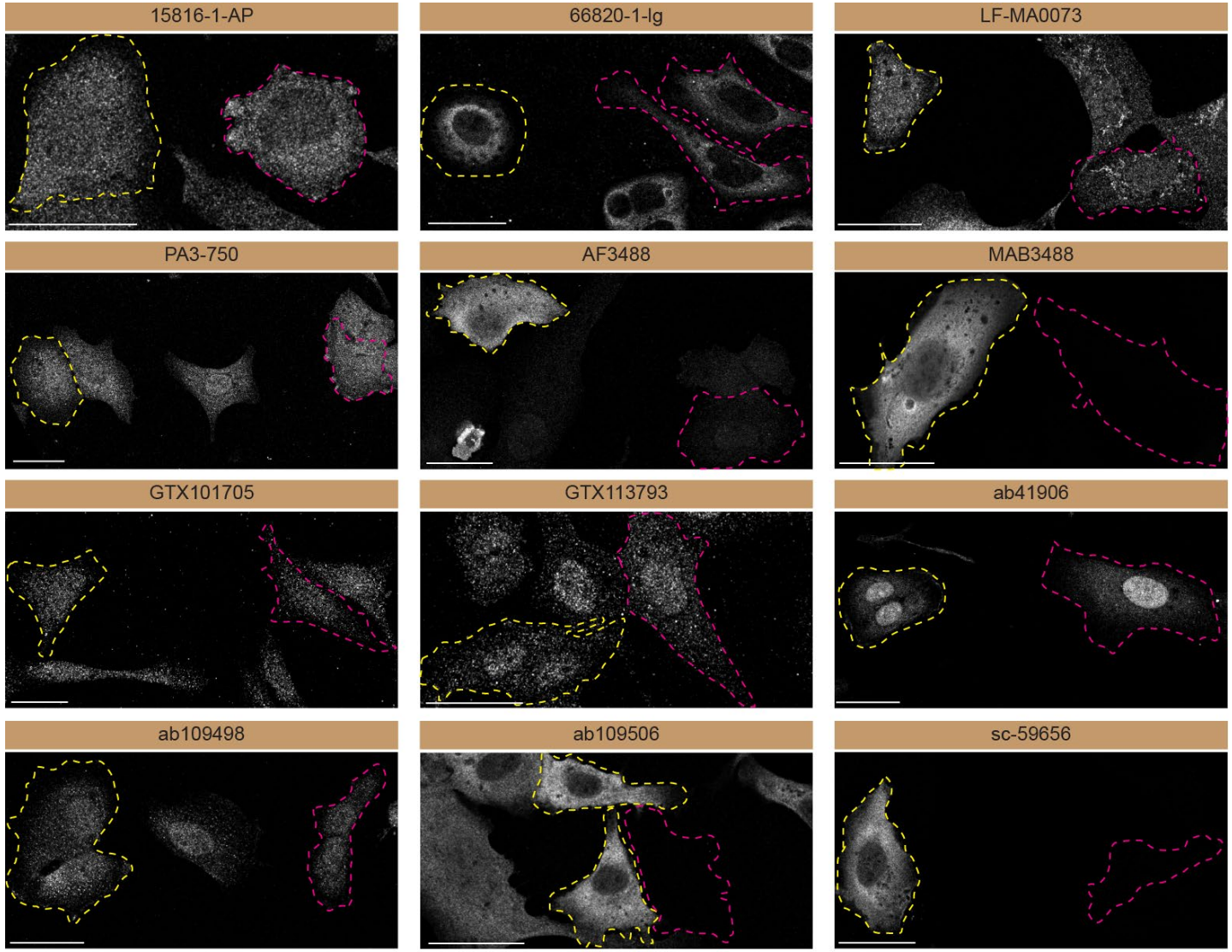

Figure 3: Peroxiredoxin-1 antibody screening by immunofluorescence

Materials and methods

Antibodies

All Peroxiredoxin-1 antibodies are listed in Table 1. Peroxidase-conjugated goat anti-mouse and anti-rabbit antibodies are from Thermo Fisher Scientific (cat. number 65-6120 and 62-6520). Alexa-647-conjugated goat anti-mouse, goat anti-rabbit and donkey anti-goat secondary antibodies are from Thermo Fisher Scientific (cat. number A21236, A21245 and A21447).

Cell lines

U2OS *PRDX1* KO clone was generated by following an open-access protocol available on Zenodo.org: https://zenodo.org/record/3875777#.X9uE11VKjIX. The gRNA sequence used to introduce a STOP codon in the *PRDX1* gene is: cttgttgacccacttggtga.

U2OS WT and KO were cultured in DMEM high-glucose (GE Healthcare cat. number SH30081.01) containing 10% bovine calf serum (GE Healthcare cat. number SH30072.03), 2 mM L-glutamate (Wisent cat. number 609065, 100 IU penicillin and 100 µg/ml streptomycin (Wisent cat. number 450201).

Immunoblot

U2OS parental and *PRDX1* KO cells were collected in RIPA buffer (50 mM Tris pH 8.0, 150 mM NaCl, 1.0 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitor. Lysates were sonicated briefly and incubated 30 min on ice. Lysates were spun at ~240,000xg for 15 min at 4°C and equal protein aliquots of the supernatants were analyzed by SDS-PAGE and immunoblot.

Immunoblots were performed with large 4-15% gradient polyacrylamide gels and transferred on nitrocellulose membranes. Proteins on the blots were visualized with Ponceau staining which is scanned to show together with individual immunoblot. Blots were blocked with 5% milk for 1 hr, and antibodies were incubated O/N at 4°C with 5% bovine serum albumin in TBS with 0,1% Tween 20 (TBST). Following three washes with TBST, the peroxidase conjugated secondary antibody was incubated at a dilution of ~0.2 µg/ml in TBST with 5% milk for 1 hr at room temperature followed by three washes with TBST. Membranes are incubated with ECL from Pierce (cat. number 32106) prior to detection with HyBlot CL autoradiography films from Denville (cat. number 1159T41).

Immunoprecipitation

Antibody-bead conjugate were prepared by adding 1.0 µg of antibody to 500 ul of PBS with 0,01% triton X-100 in a microcentrifuge tube, together with 30 µl of protein A- (for rabbit antibodies) or protein G- (for mouse antibodies) Sepharose beads. Tubes were rocked O/N at 4°C followed by several washes to remove unbound antibodies.

U2OS were collected in HEPES buffer (20 mM HEPES, 100 mM sodium chloride, 1 mM EDTA, 1% Triton X-100, pH 7.4) supplemented with protease inhibitor. Following 30 min on ice, lysates were spun at 110,000xg for 15 min at 4°C. One ml aliquots at 1 mg/ml of lysate were incubated with an antibody-bead conjugate for ~2 hrs at 4°C. Following centrifugation, the unbound fractions were collected and beads were subsequently washed three times with 1 ml of HEPES lysis buffer, and processed for SDS-PAGE and immunoblot.

Immunofluorescence

U2OS cells (parental and *PRDX1* KO) were transfected with GFP and mCherry respectively. At 48 hrs post transfection, both cell lines were plated on glass coverslips as a mosaic and incubated for 24 hrs. Cells were fixed in 4% PFA (in PBS) for 15 min at room temperature and then washed 3 times with PBS. Cells were permeabilized in PBS with 0,1% Triton X-100 for 15 min at room temperature and blocked in PBS with 5% BSA, 5% goat serum and 0.01% Triton X-100 for 30 min at room temperature. Coverslips were incubated face down on a 50 µl drop (on paraffin film in a moist chamber) of IF buffer (PBS, 5% BSA, 0,01% Triton X-100) containing the primary Peroxiredoxin-1 antibodies O/N at 4°C. Cells were washed 3 × 10 min and incubated with corresponding Alexa Fluor 647-conjugated secondary antibodies diluted 1:2000 in IF buffer for 1 hr at room temperature. Cells were washed 3 × 10 min with IF buffer and once with PBS. Coverslips were mounted on a microscopic slide using fluorescence mounting media (DAKO).

Imaging was performed using a Leica SP8 laser scanning confocal microscope equipped with a 40x oil objective (NA = 1.30) and HyD detectors. Acquisition was performed using Leica Application Suite X software (version 3.1.5.16308). Analysis was done using Image J. All cell images represent a single focal plane. Figures were prepared for publication using Adobe Photoshop to adjust contrast, apply 1 pixel Gaussian blur and then assembled with Adobe Illustrator.